Structural Characterization of Four Ribose-methylated Nucleosides from the Transfer RNA of Extremely Thermophilic Archaebacteria

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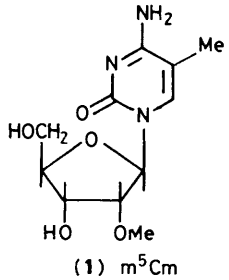
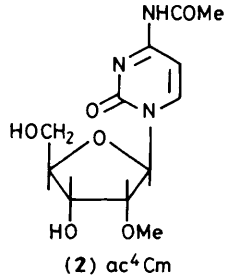
Structures of the ribose-methylated nucleosides 5,2′-*O*-dimethylcytidine (**1**), N^4-acetyl-2′-*O*-methylcytidine (**2**), 2-thio-2′-*O*-methyluridine (**3**), and N^2,N^2,2′-*O*-trimethylguanosine (**4**) from the transfer RNA of *Sulfolobus solfataricus*, *Thermoproteus neutrophilus*, and *Pyrodictium occultum* have been established, and verified by chemical synthesis.

The nature and extent of structural modifications in nucleosides from archaebacterial transfer RNA (tRNA) reflect two factors: (i) adaptation to conditions of growth, which in some cases are extreme,[1] and (ii) phylogenetic differences[2] between archaebacteria and the other two primary kingdoms, eukaryotes and the eubacteria. Approximately 65 nucleosides are presently known in tRNA from all sources, 19 of which have been found in archaebacteria and which tend to follow the modification patterns found in eukaryotes rather than eubacteria.[3]

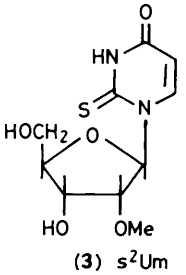
Transfer RNA has been studied from three extremely thermophilic archaebacteria, *Sulfolobus solfataricus* (optimal growth 87 °C),[4] *Thermoproteus neutrophilus* (88 °C),[5] and *Pyrodictium occultum* (105 °C), the latter organism being the highest temperature form of life presently known.[6] The structures of four ribose-methylated nucleosides (**1**)—(**4**), have been determined, primarily by mass spectrometry; these compounds are thus far unique to the extreme thermophiles. Following digestion of unfractionated tRNA to ribonucleosides by nuclease P_1 and bacterial alkaline phosphatase, enzymatic hydrolysates were examined by thermospray liquid chromatography–mass spectrometry (l.c.–m.s.).[7] Structurally diagnostic ions[7] [MH^+, BH_2^+ (B = base), (sugar − H)NH_4^+]; and elution times in the h.p.l.c. system of Buck *et al.*[8] (column temperature 31 °C) were: (**1**), *m/z* 272, 126, 164; 17.5 min; (**2**), *m/z* 300, 154, 164; 22.9 min; (**3**), *m/z* 275, 129, 164; 24.3 min; (**4**), *m/z* 326, 180, 164; 27.8 min.



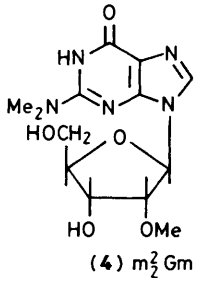
(**1**) m^5Cm



(**2**) ac^4Cm



(**3**) s^2Um



(**4**) m_2^2Gm

Compounds (**1**)—(**4**), not previously synthesized, were prepared from the corresponding ribonucleosides by methylation of O-2′ with diazomethane [(**1**)[9], (**4**)[10]], by acetylation[11] of 2′-*O*-methylcytidine (**2**), or by O-2′ methylation of O^2,5′-anhydrouridine followed by treatment with H_2S (**3**).[12] The elution positions in reversed-phase h.p.l.c.[8] of synthetic (**1**)—(**4**) corresponded to those of the natural nucleosides as measured by l.c.–m.s. with selected ion monitoring. Additionally, (**1**) was clearly distinguished from the isomeric N^4,2′-*O*-dimethylcytidine by the h.p.l.c. retention time of the latter (17.2 min). The 3,2′-*O*-dimethyl isomer was excluded by carrying out l.c.–m.s. of an *S. solfataricus* isolate with D_2O h.p.l.c. mobile phase, which showed exclusively *m/z* 277 for MD^+ [corresponding to structure (**1**)] rather than *m/z* 276, following deuterium exchange. Compound (**4**) was rigorously characterized by electron ionization mass spectrometry of its trimethylsilyl derivative[13] which was indistinguishable from that of the synthetic material: M^+, *m/z* 541.2584 (29% rel. int., 541.2572 calc. for $C_{22}H_{43}N_5O_5Si_3$); M − Me, 526 (7.4); B (base) + 1′-CH + 2′-CHOMe, 308 (17); sugar − H, 290 (1.6); B + CH_2O, 280 (20); B + H, 251 (41); B + H − MeN, 222 (17), Me_3Si, 73 (100).

Compounds (**1**)—(**4**) were each found in tRNA from all three organisms, with the exception of (**2**) and (**4**) which were not detected in *S. solfataricus*, and *P. occultum*, respectively. Nucleoside N at position 26 in the initiator tRNA of *S. acidocaldarius* was earlier presumed to be (**4**) (no data given),[14] an assignment indirectly supported in the present study by the characterization of (**4**) in the *solfataricus* strain. L.c.–m.s. experiments have also revealed the presence of a number of nucleosides known in eubacterial and eukaryotic tRNA, but not previously reported in archaebacteria,[3] identified by their thermospray mass spectra and h.p.l.c. elution times: 2-thiouridine, *m/z* 261, 129; 15.4 min; 2′-*O*-methyladenosine, *m/z* 282, 136; 22.2 min; N^6-methyladenosine, *m/z* 282, 150; 24.3 min; *N*-[(9-β-D-ribofuranosyl-2-methylthiopurin-6-yl)carbamoyl]threonine, *m/z* 314, 182, 120; 27.4 min; N^6,N^6-dimethyladenosine, *m/z* 296, 164; 32.2 min. With the exception of 2-thiouridine, detected only in *S. solfataricus*, these constituents were found in all three organisms, and demonstrate that the diversity of structural modification in archaebacterial tRNA is greater than previously thought.

In thermophilic eubacteria, pyrimidine thiation at position-54 of tRNA is known to be associated with increased thermal stabilization,[15,16] while there is speculation that the increasing extent of ribose methylation with growth temperature provides protection against nuclease attack.[17,18] The extent to

which the present findings are relevant to these issues must await determination of their sequence locations in isoaccepting tRNAs.

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